Original Article

DOI: 10.7860/JCDR/2024/67111.18949

Radiology Section

Evaluation of Cytogenetic Damage Induced during Hysterosalpingography Procedure: A Cross-sectional Study

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ABSTRACT

Introduction: Hysterosalpingography (HSG) is a radiographic procedure used to visualise the fallopian tubes and uterine cavity by injecting a contrast medium, commonly employed for diagnosing infertility cases. However, due to the associated radiographic and fluoroscopic exposure, there is an increased risk of chromosomal damage to the gonads.

Aim: To investigate the impact of radiographic and fluoroscopic exposure on chromosomal integrity in peripheral blood lymphocytes using the Cytokinesis Blocked Micronucleus (CBMN) assay.

Materials and Methods: This cross-sectional time-bound study included 10 patients (n=10) scheduled for HSG procedures at the Department of Radiology, Justice KS Hegde Hospital, Mangaluru, Karnataka, India from April 2021 to March 2022. Following radiation exposure, the CBMN assay was conducted to evaluate chromosomal damage in both test and control group blood samples. Blood samples from healthy donors were divided into three cryo vials, with one serving as the control and the remaining two exposed to radiation at the entrance and exit areas during the HSG procedure. The data were expressed as mean±Standard Deviation (SD). A p-value <0.05 was considered statistically significant. The analysis was performed using Statistical Package for Social Sciences (SPSS) version 20.0.

Results: A statistically significant (p<0.05) increase in biological damage was observed when comparing the Micronucleus (MN) frequency of the test groups (entrance=0.022±0.005 and exit group=0.0172±0.005) with the control group (0.006±0.003). Additionally, a decrease in MN frequency was noticed within the test groups, particularly in the exit group, although the results were not statistically significant (p=0.066).

Conclusion: The HSG is an effective clinical diagnostic technique. However, the present study indicates a potential risk of chromosomal damage associated with the procedure, emphasising the need for judicious use of radiation exposure during HSG.

Keywords: Biological effects, Cytokinesis blocked micronucleus, Fallopian tubes

INTRODUCTION

The HSG is a radiographic procedure used to diagnose the condition of the fallopian tubes and uterine cavity by introducing radiopaque contrast media through the cervical canal, employing fluoroscopy or conventional X-ray techniques. It is commonly recommended for cases of primary and secondary infertility [1]. During the procedure, radiographic and fluoroscopic exposures are focused on the urogenital region of the patient [2]. The highest radiation dose is delivered to the uterus and ovaries.

Typically, patients undergoing HSG receive an effective dose ranging from 1.2 mSv to 3.1 mSv, with radiation doses to the ovaries ranging from 2.7 mGy to 9.0 mGy. If the ovarian dose exceeds 45 mGy, appropriate analysis and implementation of radiation dose reduction measures are necessary [3]. Risks associated with radiation exposure can vary based on factors such as fluoroscopy time, the number of radiographs taken, absorbed dose, and equipment used [4]. Cumulative doses during exposure can result in higher doses in the area of interest, potentially leading to congenital disabilities or cancer [5]. The incidence of radiation-induced cancer in women undergoing the procedure increases with prolonged radiographic or fluoroscopic exposures [6]. Thus, ensuring adequate radiation protection at the individual level is of utmost importance [1].

While the exposure dose can be estimated using physical dosimeters, assessing biological damage to chromosomes provides additional information for diagnosing problems resulting from ionising radiation exposure. Therefore, the present study aimed to evaluate biological damage by examining blood lymphocytes exposed to radiation at the entry and exit areas during fluoroscopy-guided HSG procedures using the Cytokinesis Blocked Micronucleus (CBMN) assay. The CBMN assay is considered an excellent cytogenetic method for quantifying micronuclei in cultured human and mammalian cells, and it has become the standard method in the field of radiobiology [7]. The present study is the first attempt to demonstrate the effects of radiation on patients undergoing HSG procedures.

MATERIALS AND METHODS

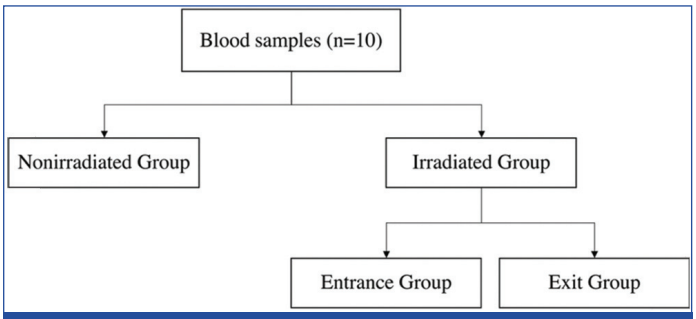
A cross-sectional time-bound study was conducted at the Department of Radiology, Justice KS Hegde Hospital, Mangaluru, Karnataka, India, from April 2021 to March 2022. The study included a total of 10 patients (n=10) scheduled for a HSG procedure. Ethical approval was obtained from the KS Hegde Medical Academy Institutional Ethics Committee (INST. EC/EC/095/2021-22). Written informed consent was obtained from all patients and blood donors participating in the study.

Inclusion and Exclusion criteria: The study included blood donors aged between 28 to 39 years who had no previous exposure to radiation within the past six months and no existing medical conditions. Patients with a history of infertility were also included. Subjects who were currently taking medication for metabolic disorders or other diseases were excluded from the study. Additionally, blood donors who had undergone radiographic examinations within the last seven days were not eligible to donate blood.

Study Procedure

Blood samples were collected in lithium-heparin vacutainers and divided into three cryo vials, each containing 1 mL of blood. These vials were placed at the entrance and exit points of the radiation

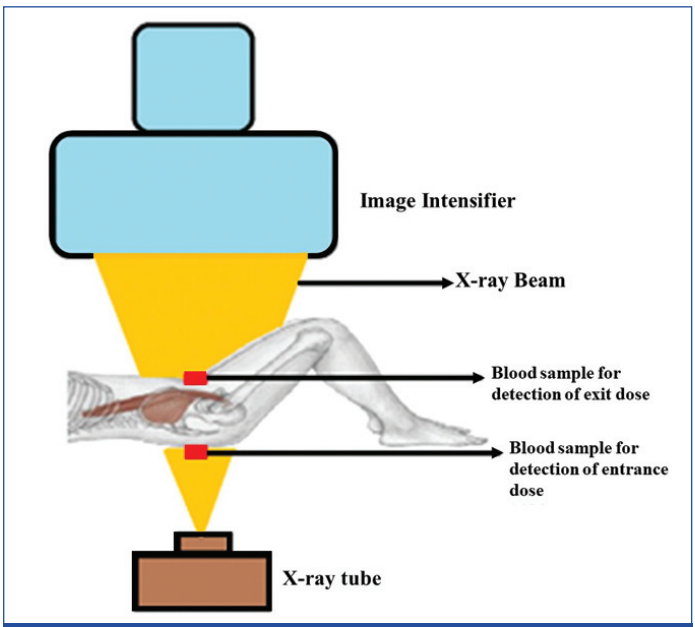
area during the HSG procedure. Two aliquoted cryo vials were placed parallel to each other, one at the dorsal part and the other at the ventral part of the patient's pelvic region, to assess the effect of radiation at the entrance and exit sites. The third vial served as the control. The flowchart grouping of the blood samples is shown in [Table/Fig-1].



[Table/Fig-1]: Flowchart depicting the grouping of the blood samples.

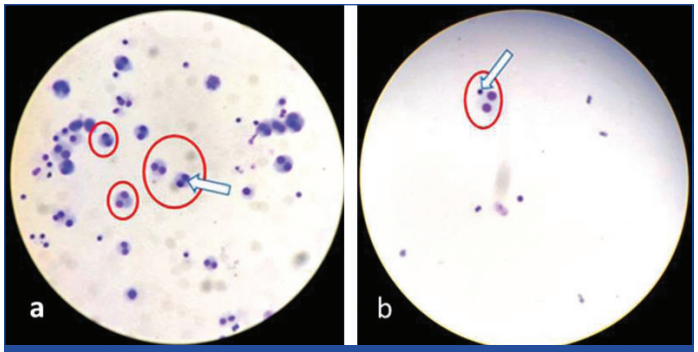
HSG Procedures

The patient was positioned in the lithotomy position, lying on their back with knees bent and feet supported on footrests [Table/Fig-2]. The radiologist performed the HSG procedure using strict aseptic precautions. An 8F Foley's catheter was advanced to the cervical of to inject the contrast. Urograffin 76% (diluted in a 1:1 ratio with sterile water) was injected under fluoroscopic guidance. The volume of contrast medium injected was 6-8 mL. Details of the patient's age, weight, height, body mass index, and exposure parameters such as tube current (mA), tube load (mAs), kilovolt peak (kVp), and time (seconds) were recorded during the procedures.



[Table/Fig-2]: Graphical representation of blood sample placement during fluoroscopy-guided HSG procedures.

Estimation of biological damage by CBMN assay: Blood cultures were initiated using RPMI-1640 (80%), FBS (20%), and mitogens such as PHA (150 µL). Cytokinesis was blocked by adding cytochalasin B (6 µg/mL) at the 44th hour. After 28 hours, the culture was transferred into 10 mL centrifuge tubes. A prechilled hypotonic solution (0.075M) was added, and the solution was centrifuged at 800 rpm for eight minutes. Cells were fixed using prechilled Carnoy's fixative (5:1) solution. Slides were prepared, stained with Giemsa stain (8%), and dried. The dried slides were visualised under the Primo Star Light-emitting Diode (LED) microscope (40X magnification). A minimum of 2000 binucleated cells were scored following the criteria outlined in the protocol published by Fenech M [8]. A microscopic image showing a) a binucleated cell and b) a binucleated cell with a MN is provided in [Table/Fig-3].



[Table/Fig-3]: Microscopic images showing: (a) A white arrow indicating a binucleated cell; (b) A white arrow indicating a binucleated cell with a MN.

STATISTICAL ANALYSIS

The data were expressed as mean±standard deviation. To compare between groups, Dunnett's multiple comparison test was used. Spearman's rank correlation coefficient was applied to compare MN entrance and exit sites with the physical parameters. A p-value <0.05 was considered statistically significant. The analysis was performed using SPSS version 20.0.

RESULTS

Parameters of radiation exposure: The radiation exposure parameters utilised during the procedure were carefully recorded. [Table/Fig-4] presents the mean±standard deviation of the tube current (mA), tube load (mAs), kilovolt peak (kVp), number of spot films, and time (seconds).

Exposure parameters	Mean±SD
Fluoroscopy mA	2.31±0.17
Radiography time (seconds)	0.04±0.00
Fluoroscopy kVp	62.50±2.27
Radiography kVp	62.50±2.27
No. of spot films	2.50±0.71
Fluoroscopy time (seconds)	73.60±42.59
Total Radiation exposure time (Radiography+Fluoroscopy)	73.64±42.59

[Table/Fig-4]: Descriptive statistics (mean±SD) for radiation exposure technical parameters.

Estimation of biological damage: As shown in [Table/Fig-5], a significant (p<0.05) increase in biological damage was observed when comparing the MN frequency of the test groups (entrance and exit groups) with the control group. However, between the test groups, a decrease in MN frequency was observed in the exit group, although the results were not statistically significant (p>0.05).

Group	Micronucleus frequency 1 (Mean±SD)	Micronucleus frequency 2 (Mean±SD)	Mean difference	Standard error of difference	p-value
Control vs. entrance group	0.006±0.003	0.022±0.005	-0.016	0.0021	<0.001
Control vs. exit group	0.006±0.003	0.0172±0.005	-0.011	0.0021	<0.001
Entrance vs. exit group	0.022±0.005	0.0172±0.005	0.005	0.0021	0.066

[Table/Fig-5]: MN frequencies in various groups.
MN frequency 1,2 represents the respective groups mentioned in the column "Group Name". Dunnett's multiple comparison test was used

Correlation of MN frequency with number of spot films and total exposure time: Spot film radiographs, which involve rapid radiation exposure, are taken during the HSG procedure instead of plain radiography. Given the continuous radiation exposure during fluoroscopy, the total exposure time was noted. Spearman's correlation analysis was conducted to evaluate the

correlation between MN frequency (entrance and exit groups) and the number of spot films as well as the total exposure time. The results indicated no significant correlation between them (p>0.05) [Table/Fig-6].

	Control MN frequency		Entrance MN frequency		Exit MN frequency	
	Spearman's ratio	p-value	Spearman's ratio	p-value	Spearman's ratio	p-value
No. of spot films	-0.126	0.730	-0.198	0.583	-0.3235	0.363
Total exposure time	0.171	0.637	0.067	0.854	0.1398	0.699

[Table/Fig-6]: Correlation of MN frequency with the number of spot films and total exposure time.

DISCUSSION

Assessment of physical doses based on different age ranges has been conducted in many studies [6,9,10]. As the HSG procedure is commonly performed in individuals between 20 to 40 years of age, representing the childbearing age, the present study selected subjects within this age group. Performing the procedure in the posteroanterior projection has been found to be highly efficient in reducing organ dose compared to the anteroposterior projection [1]. Previous research has shown that the use of posteroanterior projection can result in a reduction of ovarian dose by about 60-75% and uterine dose by 30-40% at a kVp range of 70-120 kVp [11,12]. In the study department, the HSG procedure is routinely performed in the posteroanterior projection to minimise radiation dose.

Assessment of entrance surface dose has been carried out in many studies using Thermoluminescent Dosimeters (TLD) placed in the pubic region during the HSG procedure [Table/Fig-7] [6,9,10,13,14]. The present study aimed to evaluate biological damage using the CBMN assay. The number of radiographic spot films obtained in the present study was lower compared to previous studies [6,9,13]. The present study utilised spot films for scout images and two images displaying fallopian tubal filling and peritoneal spillage. The spot films were obtained using low kilovoltage and high tube load exposures. In contrast, Perisinakis K et al., conducted a study where the field was vice-versa [6]. The variation in exposure parameters contributed to an increase in MN frequencies, indicating an increase in biological damage.

Although the radiation-associated risks in an average HSG procedure are considered allowable, it is important to emphasise measures to reduce unnecessary irradiation in women of childbearing age who desire pregnancy. Dose reduction methods, such as minimising unnecessary fluoroscopic screening and the number of spot films obtained, should be encouraged. The procedure should be conducted by a trained radiologist with a restricted field of view to the area of requirement. Other non ionising radiation methods like Magnetic Resonance (MR)-HSG, which provide three-dimensional information without the use of ionising radiation, should also be considered [15]. Nonetheless, this is the first study attempting to show the effects of radiation in patients undergoing HSG procedures. Further in-vivo studies with physical dosimetry and additional biological parameters are needed to further expand knowledge in this area.

In the present study, a comparison was made between the control MN frequency and the entrance MN frequency using the CBMN assay. Statistical significance (p<0.05) was observed, indicating increased biological damage in the entrance MN frequency. As radiation passes through tissue, the dose absorbed progressively decreases, resulting in higher doses at the entrance area compared to the exit area [16]. While no studies have reported estimating exit dose using physical or bio dosimetry during gynaecological procedures, the present study showed statistical significance (p<0.05) between the control MN frequency and the exit MN frequency, indicating that X-rays exiting the patients can still cause biological damage. However, no statistical significance was observed between the entrance and exit MN frequencies in the present study, suggesting similar biological damage in the entrance and exit blood samples.

During the HSG procedure, it is essential to control the radiation dose received by the patient to minimise radiogenic risks to the gonads. The results of earlier published articles were compared with the current study [Table/Fig-8]. Practices such as appropriate use of higher kVp, lower mA, and increased X-ray beam filtration should be implemented [1]. The role of fluoroscopic screening in the present study was mainly to monitor the flow of contrast media. The mean fluoroscopy screening time during the HSG procedure in our study was less than the time mentioned in one study [14] and more than the time reported in similar studies [6,9,10,13].

Authors	Year and place of the study	Age range of patients (years)	Mean fluoroscopy time (minutes)	Number of radiographic spot films	ESD (mGy)	Type of dosimetric study	Assessment of MN frequency
Perisinakis K et al., [6]	2003 Greece	18-39	0.3	3.2	9.7	Physical dosimetry	No
Gregan ACM et al., [10]	1998 London	24-39	0.3	2	14.6	Physical dosimetry	No
Fife IAJ et al., [13]	1994 London	-	0.7	3.6	13.3	Physical dosimetry	No
Karande VC et al., [14]	1997 Chicago	-	1.3	0	14	Physical dosimetry	No
Fernández JM et al., [9]	1996 Spain	26-42	0.5	7	-	Physical dosimetry	No

[Table/Fig-7]: Comparison of results of the previously conducted studies using dosimeter [6,9,10,13,14].

Technical parameters	Perisinakis K et al., [6]		Present study	
	Fluoroscopy	Radiography	Fluoroscopy	Radiography
Tube current (mA)	2.8±0.3	-	2.31±0.2	-
Kilovoltage (kVp)	97±5	88±1	62.50±2.3	62.50±2.3
Tube load (mAs)	-	17±12	-	33.20±2.7
Fluoroscopy time (minutes)	0.3±0.2	-	1.22±0.71	-
Number of radiographs	-	3.2±0.2	-	2.50±0.71

[Table/Fig-8]: Comparison of technical parameters of the present study with previously conducted study [6].

Limitation(s)

Future studies can be undertaken to compare physical dosimetry and assess the biological effects to predict radiogenic risks associated with biological damage. However, the limitations of the present study include the sample size and the absence of physical dosimetry parameters such as TLD badge data.

CONCLUSION(S)

The HSG procedure carries a risk of chromosomal damage, which should be taken into consideration to minimise radiation exposure. The observed increase in MN frequency due to radiation highlights the need for implementing precautionary regulatory measures to ensure patient safety during the procedure.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Aug 21, 2023
- Manual Googling: Nov 02, 2023
- iThenticate Software: Nov 16, 2023 (4%)

ETYMOLOGY: Author Origin

EMENDATIONS: 6

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Aug 18, 2023**
Date of Peer Review: **Oct 20, 2023**
Date of Acceptance: **Nov 19, 2023**
Date of Publishing: **Jan 01, 2024**